the downstream domains that lead into the transmembrane helix. To address this issue, we have combined multiscale molecular dynamics (MD) simulations with biophysical assays to study the extracellular region of EphA2 upstream of the transmembrane helix, the fibronectin domain 2 (FN2). A number of novel insights emerged: (i) the FN2 domain can interact with the membrane, (ii) this domain preferentially interacts with anionic lipids, (iii) this preference is maintained through a positively charged surface of the protein, including K441 and R443. The study allows us, for the first time, to combine the recently emerged X-ray crystallography with NMR models of the transmembrane region. We present a full atomistic model of an Eph signalling cluster (extracellular regions and transmembrane regions) assembled at a model cell membrane composed of a lipid mixture. This work is a significant step in understanding the formation of Eph signalling clusters and paves the way towards understanding the assembly of full length Eph receptor platforms at the surface of cells.

1567-Pos Board B297

Cationic Lipid and Bacterial Lipopolysaccharides Both Activate Toll-Like Receptor 4 Pathways via Different Binding Regions

Caroline Lonez[1,2], Kate Irvine[2], Monique Gangloff[2], Malvina Pizzuto[1], Boris Schmidt[1], Benjamin Caroyez[1], Michel Vandenbranden[1], Clare Bryant[2], **Jean-Marie Ruysschaert**[1].
[1]Université Libre de Bruxelles, Brussels, Belgium, [2]University of Cambridge, Cambridge, United Kingdom.

DiC14-amidine is a cationic lipid which induces pro-inflammatory cytokine secretion in immune cells upon interaction with the Toll-like receptor 4 (TLR4)/Myeloid Differentiation factor-2 (MD-2) membrane bound-complex, the natural sensor of bacterial lipopolysaccharides (LPS) [1-3].
The aim of the present work is to characterize the interaction between diC14-amidine and the TLR4/MD2 receptor complex. Taking advantage of the species-dependent activity of TLR4 agonists [4], we compared the TLR4 agonist activity of diC14-amidine in four different species in order to map domains in TLR4 and MD2 that are important for diC14-amidine TLR4-agonist activity. We demonstrate that, while LPS is an agonist in all species, diC14-amidine is a full agonist for human, mouse and cat receptors, but a poor agonist for horse. Using chimeric constructs made from human and horse TLR4 and single mutants, we identify two regions in the human TLR4 that modulates the agonist activity of diC14-amidine. Interestingly, these regions in TLR4 are different from the previously identified bacterial lipopolysaccharides binding domains [5]. How ligand binding to two different regions of the same receptor affects the cellular pathways is currently under investigation.
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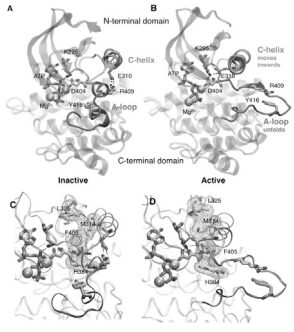
1568-Pos Board B298

Activation Pathways of Kinases Reveal Intermediate States as Novel Targets for Drug Design

Diwakar Shukla[1], Benoit Roux[2], Vijay S. Pande[1].
[1]Stanford University, STANFORD, CA, USA, [2]University of Chicago, Chicago, CA, USA.

Unregulated activation of kinases leads to aberrant signaling, uncontrolled growth, and differentiation of cancerous cells. Reaching a complete mechanistic understanding of the large scale conformational transformations underlying the activation of kinases could greatly help in the development of therapeutic drugs for the treatment of these pathologies. In princi- ple, the nature of conformational transition could be modeled by in silico via atomistic molecular dynamics simulations, although this is very challenging due to the long timescales (100s of μs) associated with activation. In this study, we employ a computational paradigm that couples transition pathway generation techniques and Markov State Model (MSM) based massively (total simulation time of ~3 milliseconds) distributed simulations for mapping the conformational landscape of several key tyrosine kinases. The computations provide the thermodynamics and kinetics of kinase activation for the first time, and help identify key intermediates along the activation pathway. Furthermore, the presence of a novel allosteric sites in an intermediate states of kinases that could be potentially utilized for drug design is predicted.



1569-Pos Board B299

Towards the Rational Design of Opioids with Desired Binding Kinetics

Sebastian Schneider, Davide Provasi, Marta Filizola.
Department of Structural and Chemical Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Opioid receptors are classical pharmacological targets and the object of intense research towards improved analgesics with reduced side effects. High-resolution X-ray crystal structures of all four opioid receptor subtypes - the mu (MOP), kappa (KOP), delta (DOP), and nociceptin (NOP) opioid receptors - have recently provided direct information about the molecular determinants of opioid binding affinity. Yet, little information is available about the kinetics of opioid-receptor binding, which may be as important as, or even more important than, affinity for the rational design of efficacious opioid-based therapeutics.
By accelerating transitions between low-energy states, enhanced molecular dynamics techniques are especially suited to gain atomic-level insight into opioid receptor recognition and binding. Here, we used multiple-walker well-tempered metadynamics to investigate the binding of classical opioid receptor ligands to the mouse MOP receptor. We find that the first interaction between the ligand and MOP involves residue D218 in the extracellular loop 2 and residues of transmembrane (TM) helix 2. Analysis of the lowest-energy binding pathways reveals an additional intermediate metastable state in which the ligand positions itself in a region that is partially occupied by JDTic in the corresponding KOP crystal structure, forming interactions with residues in TM2 and TM3.
We used the free-energies resulting from our simulations to derive estimates of binding affinity and transition rates along the characterized opioid binding pathways, as well as ligand on- and off-rates. Given the reasonable agreement between the calculated estimates and published experimental values, and the provided powerful insights into molecular determinants of opioid-receptor binding kinetics, these types of simulation hold great promise for the rational design of opioids with desired binding kinetics.

1570-Pos Board B300

Computational Prediction of the Class a GPCR Active State Conformations

Sijia S. Dong, Ravinder Abrol, William A. Goddard III.
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA.

There has been a great need for computational prediction of G-protein coupled receptor (GPCR) structures, especially those in their active states, in order to assist drug discovery. However, the active state conformation prediction is challenging not only because of the lack of homology templates derived from the existing active state X-ray structures, but also because of the high energy nature of the active conformations. In addition, experimental evidence suggests that a GPCR can have many different active states, but the existing several crystal structures usually only capture one of those states for each GPCR. Here we present a method to make the GPCR active state structural prediction possible, and our method can discover a number of active states for each GPCR. Instead of the traditional homology modeling, we used a template from mixed sources and sampled a discrete set of orientations of the seven transmembrane helices of the GPCR to locate structures that are likely to be in the active-state valley on the energy surface. Next, we did a local conformational sampling to find structures at the local minima of the active-state potential energy valleys. We have benchmarked the method with human β_2 adrenergic receptor, which has both its active and inactive state structures crystalized. Then we applied the method on a GPCR with unknown structure, the human somatostatin receptor subtype 5 (hSSTR5). Docking of agonists and antagonists to the predicted active and inactive state structures of hSSTR5 gave the expected result that antagonists favor the inactive state structures, while the agonists could not distinguish the inactive and active state structures without the presence of G proteins. In the end, we were able to build a model picture of hSSTR5 function consistent with experimental findings.

1571-Pos Board B301

Exploring the Structure and Dynamics of All-Atom Models for the Plexin Transmembrane Receptor Bound to GTPases and to Lipid Bilayer

Liqun Zhang, Matthias Buck.
Physiology and Biophysics, Case Western Reserve University, Solon, OH, USA.

Plexins are transmembrane receptors that receive guidance cues (such as binding of Semaphorin ligands) and are activated by them, functioning in cell migration processes in neuronal and cardiovascular development, but also in cancer metastasis. Plexins are unique, as they are the first example of a receptor that interacts directly with small GTPases, a family of proteins that are essential for cell motility and proliferation/survival. We previously determined the